Vitamin B12 Deficiency: an Update for the General Paediatrician

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Abstract

Vitamin B12 deficiency is an important and possibly under recognised cause of neurological morbidity in infants. The causes of infantile vitamin B12 deficiency are heterogeneous, ranging from dietary deficiency in a breast feeding mother to specific inborn errors of metabolism.

In this brief review we discuss the clinical presentations of vitamin B12 deficiency, provide a practical approach to the investigation and management of infantile vitamin B12 deficiency, and consider specific neurometabolic reasons why the infantile central nervous system is vulnerable to irreversible damage from Vitamin B12 deficiency.

Keywords: Vitamin B12; Cyanocobalamin; Giardia lamblia

Introduction

Vitamin B12 or Cobalamin (Cbl) is a water-soluble vitamin. In the human body it occurs in 3 different forms: the natural form hydroxocobalamin (OH-Cbl) and in its two active forms methylcobalamin (Me-Cbl) and adenosylcobalamin (Ado-Cbl). Cyanocobalamin is a commercially available pharmacological form.

Cobalamin is synthesised by microorganisms present in the environment and in the intestines of animals. Common dietary sources include meat fish and dairy products or food that has been fermented. The Recommended Daily Allowance (RDA) ranges from 0.4 mcg/day for age< 6 months to 2.4 mcg/day for adults [1]. Cobalamin deficiency may be more common than previously recognised.

Data from the NHANES III study of 3766 US children aged 4-19years identified 1/1255 children with levels<100 pg/ml and 1/200 children <200 pg/ml. B12 Levels<100 pg/ml were considered to be indicative of clinical deficiency for the purpose of the survey (100 pg/ml=74 pmol/L) [2]. In less developed countries, B12 deficiency may be even higher. A study of rural Mexican children showed 22% had serum B12 levels <103 pmol/L (140 pg/ml), a quarter of these children were thought to have malabsorption due to Giardia lamblia infection or bacterial overgrowth [3].

Key Points

a. An elevated propionylcarnitine (C3) on Extended Newborn Screening (ENBS) can be a marker for Vitamin B12 deficiency.

b. Vitamin B12 deficiency in breast feed infants is most commonly due to maternal deficiency.

c. Maternal dietary Vitamin B12 deficiency can occur in non-vegan mothers.

d. Vitamin B12 deficiency has heterogeneous range of neurological presentations.

e. Elevations in Methylmalonic Acid (MMA) and Homocysteine (Hct) are more sensitive functional markers of vitamin B12 status than Mean Corpuscular Volume (MCV).

f. Prompt identification of and treatment of infantile Vitamin B12 deficiency is important to prevent irreversible neurological sequelae.

g. Vitamin B12 deficiency should be considered in every infant with developmental delay and Hypotonia.

Cobalamin Metabolism

Cbl is involved in two essential cellular reactions (Figure 1). Firstly Me-Cbl is required for the methylation of methionine to homocysteine paired with the demethylation of methyltetrahydrofolate to tetrahydrofolate. Secondly Ado-Cbl is a cofactor for the conversion of methylmalonyl-CoA to succinyl-CoA. When these cofactors are deficient homocysteine and/or methylmalonate accumulate.

Me-Cbl is a cofactor for methionine synthase, catalysing the conversion of homocysteine to methionine in the cytoplasm and additionally participating in the recycling of folate. S-Adenosyl methionine is important as a universal methyl group donor in more than 100 organic reactions. These reactions are particularly important for DNA synthesis.

The second reaction, occurring in the mitochondria, is the conversion of L methylmalonyl CoA to succinyl-CoA. Methylmalonyl CoA is formed in the of catabolism of branched chain amino acids,
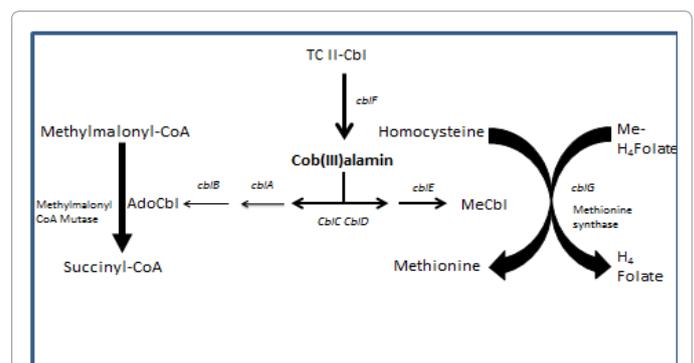

Figure 1: Metabolic reactions catalysed by the active forms of cobalamin.

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cholesterol and odd chain fatty acids and this is the essential pathway for its metabolism. Decreased activity of methylmalonyl -CoA mutase results in accumulation of methylmalonyl-CoA and its upstream metabolite, prionyl-CoA. Propionylcarnitine (C3) is produced in the conversion of excess prionyl-CoA. C3 is included in tandem mass spectrometry based Extended Newborn Screening (ENBS) programmes although its sensitivity to detect all neonates with B12 deficiency is unknown. Its sensitivity will depends on whether C3 levels reach the local laboratories assigned cut-off when the ENBS sample is collected.

Maternal Influences on Infantile Vitamin B12 Deficiency and Extended Newborn Screening

The causes of maternal B12 deficiency includes strict vegan diet, poverty and malnutrition, occult pernicious anaemia, previous gastric bypass surgery, and short gut syndrome [4-10]. Maternal B12 deficiency can be subclinical, they may not be anaemic and their vitamin B12 levels normal or low-normal [11]. Maternal vitamin B12 deficiency, while associated with a strict vegan diet, can also been seen in mothers with sub-optimal nutrition especially those from a lower socioeconomic status, hence the importance of the Paediatrician taking a maternal dietary history in cases of infantile vitamin B12 deficiency.

During pregnancy the placenta actively concentrates cobalamin in foetus resulting in foetal serum levels twice those of maternal serum [12]. Under normal circumstances the term neonate has sufficient stores to last for 6-12 months [13]. Cobalamin deficiency in newborns therefore reflects deficiency in the mother, and an elevated C3 detected on ENBS can be a marker of maternal vitamin B12 deficiency [14,15]. Specific cases of maternal dietar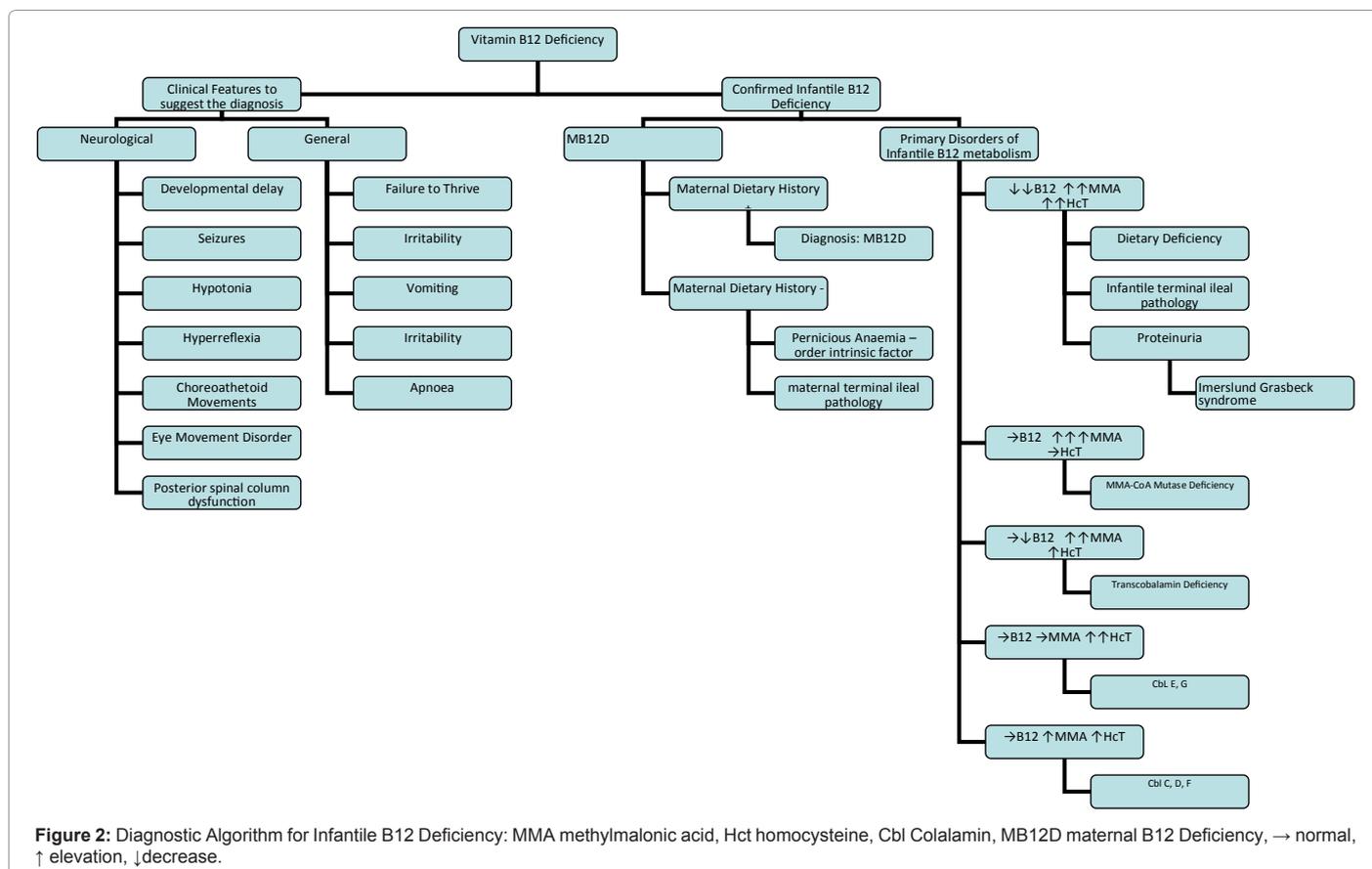y B12 deficiency and subclinical maternal pernicious anaemia have been reported in the medical literature after identification of an elevated C3 on ENBS in their newborn [16-18]. However the natural history of the rise of C3 in deficient infants is unclear. A negative newborn screen should not be relied upon to rule out Vitamin B12 deficiency [14].

It has been shown that mothers with low cobalamin levels have high Homocysteine (Hct) and Methylmalonate (MMA) levels and predictably low cobalamin and high Hct/MMA levels in their newborns, additionally higher birth number also increases the risk of low cobalamin status in these mothers [16,19]. In infants less than 6 months of age, the MMA concentration is inversely related to cobalamin concentrations [19]. This observation underlies the importance of cobalamin as a cofactor for MMA and Hct metabolism (Figure 1).

Cobalamin deficiency is especially important for mothers who choose to breast feed. On average, the cobalamin concentration in breast milk is 0.42 mcg/L [20]. Breast milk B12 concentrations have been found to be lower in women consuming a strict vegetarian diet compared to omnivorous women (0.23 ± 0.09 mcg/L vs. 0.38 ± 0.08 mcg/L). Infants fed breast milk containing less than 0.36 mcg/L had elevated methylmalonate levels. Additionally the milk B12 concentration was inversely proportional to the length of time the vegetarian diet was consumed [21]. Exclusively breast fed infants of deficient mothers are most at risk as most commercially available infant formulas are fortified with cobalamin.

Inborn Errors of Cobalamin Synthesis

Numerous autosomal recessively inherited inborn errors of Cbl transport or metabolism are known to exist. They exhibit molecular

Figure 2: Diagnostic Algorithm for Infantile B12 Deficiency: MMA methylmalonic acid, Hct homocysteine, Cbl Colalamin, MB12D maternal B12 Deficiency, → normal, ↑ elevation, ↓decrease.

and clinical heterogeneity. However; the following simple algorithms can be utilised to direct the clinician towards the metabolic pathway affected (Figure 2);

a. Low B12 level, elevated MMA and Hct indicate a B12 deficiency or transport problem

b. Normal B12 level, elevated MMA and normal Hct indicate a block in Ado-Cbl metabolism

c. Normal B12 level, normal MMA and elevated Hct indicate a block in Me-Cbl metabolism

Clinical Manifestations of Vitamin B12 Deficiency in Infants and Children

Early manifestations of cobalamin deficiency in infancy are non specific and thus can lead to a delayed diagnosis; they include failure to thrive, vomiting, irritability, weakness and refusal to weaned [22].

Neurological manifestations are common in infantile B12 deficiency, perhaps reflecting the importance of vitamin B12 in normal brain development and maturation. Reported infantile neurological manifestations of B12 deficiency include poor feeding, Hypotonia, developmental delay, developmental regression, eye movement abnormalities, irritability, chorea, tremor and seizures [23-25]. A key observation is that infants with neurological manifestations of B12 deficiency can still have normal haematological parameters including MCV [15,26], however the biochemical perturbations of elevated MMA and Hct associated with Vitamin B12 Deficiency precede the haematological and clinical manifestations [15,16].

Cobalamin deficiency in older children may present with paraesthesia, ataxia, abnormal movements, glossitis and personality change. Abnormal pigmentation of the dorsum of the fingers, toes and in the axillae, arms and medial thighs has been reported in older children with severe cobalamin deficiency [8,27,28]. Common reported signs are Hypotonia, hyperreflexia and choreoathetoid movements [11]. The presence of seizures at diagnosis seems to predict more severe developmental outcome [10]. Although not all authors have reported

long term neurodevelopment follow up of their cases, poor infant developmental outcomes occur in 38% of pernicious anaemia mothers and 50% of vegan mothers [8,29].

Treatment with parenteral hydroxocobalamin results in dramatic improvement in abnormal movements, cessation in seizures, and improved energy and appetite. Cerebral atrophy as demonstrated on MRI has been shown to reverse [8]. Abnormal movements can appear transiently during treatment in some infants- the cause is unknown.

Pathogenic Mechanisms of B12 Deficiency in Infants and Children

The infantile brain appears to be highly ssusceptible to adverse sequelae manifesting from vitamin B12 deficiency. The precise mechanism of this neurological dysfunction is unclear, but is likely to be multi-factorial in aetiology, with postulations including;

a. Interference with normal myelination

b. Epigenetic causes from deranged s-adenosylmethionine production

c. Aberrant cytokine regulation

The infantile brain is particularly susceptible to the myelination based mechanisms of B12 deficiency as myelination occurs mostly in the first 2 years of life, but is at its peak in the first 6 months of life. Long standing vitamin B12 deficiency has been well documented to result in delayed myelination or demyelination of the brain and spinal cord [30-32]. Deficiency of Ado-Cbl results in impaired enzymatic activity of methylmalonyl-CoA mutase, which in turn leads to an accumulation of prionyl-CoA. This in turns leads an accumulation of C15 and C17 fatty acids into the nerve sheaths resulting in altered myelin with reduced components of phospholipids, sphingomyelin and ethanolamine [33,34]. A deficiency of Me-Cbl, leading to impaired conversion of homocysteine to methionine, ultimately reduces the conversion of methionine into the key metabolite S-Adenosylmethionine (SAM). SAM is an important methyl donor for the conversion of phosphatidylethanolamine to phosphatidylcholine; both of these lipids are key components of myelin [35]. An interesting clinical observation

Defect	Presenting age	MMA	Homocysteine	Serum cbl	Haematological feature	Neurological features	Other
Maternal B12 deficiency	infancy	Mild-moderate elevation	Mild elevation	low	Macrocytic anaemia	Developmental delay Apathy Abnormal movements seizures	Symptoms respond rapidly to treatment
Imerslund Grasbeck syndrome	1-5 years	-	-	Very low	Megaloblastic anaemia-responsive to IM B12	Reported occasionally	Low B12 absorption not corrected by IF Proteinuria
Transport defect (TCII deficiency)	1-2 months	Mild-moderate elevation	Mild elevation	normal	immature white cell precursors pancytopenia Megaloblastic anaemia	Developmental delay if diagnosed late	Chronic diarrhoea and vomiting holo Tc II low
Inborn errors							
Cbl A,B	1st month	Moderate-high	Normal	Normal	?none	Overlaps with mut0, mut-MMA	B12 responsive MMA
MMA (mut0, mut-)	Hours to weeks of life (severe form) Late onset form	Very high	Normal	normal	Neutropenia Anaemia Thrombocytopenia	Abnormal posturing Hypotonia seizures encephalopathy	Severe and recurrent metabolic acidosis Increased anion gap hyperammonemia
Cbl C, D, F	1st few months Late onset form	Mild-moderate elevation	Mild-moderate elevation	normal	Megaloblastic anaemia pancytopenia	Severe Developmental delay Hypotonia seizures	hydrocephalus ocular abnormalities Haemolytic anaemia Cardiac disease
Cbl E, G	2 years	none	Elevated	normal	Megaloblastic anaemia	Hypotonia, seizures, developmental delay, ataxia	Low methionine

Table 1: Clinical and Biochemical Summary of B12 deficiency and Inborn Errors of Metabolism associated with Cobalamin Synthesis.

Child	Mother
CBC with peripheral blood film	CBC
MCV	MCV
Serum B12, folate,	Serum B12, folate
Urine organic acids, plasma amino acids	Urinary MMA
Urine protein	Intrinsic factor antibodies
Coeliac screen	
Stool parasites	

Table 2: First line investigations in infantile B12 deficiency.

has been the rapid improvement in the central neurological signs in infants with B12 deficiency after the administration of parenteral B12, especially rapid gains in development, improvements in tone and alertness. This rapid clinical improvement cannot be attributed alone to aberrant myelination.

Reduction in the synthesis of s-adenosylmethionine, a key methyl donor for over 100 cellular enzymatic reactions, can impact on the neurological phenotype of B12 deficiency via multiple end points including; a) abnormal synthesis of proteins, lipids and neurotransmitters, b) over stimulation of N-methyl-D-aspartate receptors, c) inhibited DNA synthesis and cell division [36-39]. Reduced availability of SAM in B12 deficiency has been postulated to increase the production of the CNS Cytokine Tumour Necrosis factor-α, which may play a role in demyelination [40-42].

Practical Approach to Diagnosis and Treatment

The diagnosis of vitamin B12 deficiency requires a high index of suspicion in children as the symptoms are generally non-specific. The nutritional history from the mother, if asked, may point to nutritional deficiency. Table 1 summarises the clinical features and biochemical features of vitamin B12 deficiency and inborn errors of metabolism of Cbl synthesis. Table 2 provides a first line strategy for investigating an infant and a mother when infantile vitamin B12 deficiency has been identified. Figure 2 provides a simplified diagnostic overview to the infant with documented B12 Deficiency.

Treatment regimes depend on the cause. Severe B12 deficiency is treated with intramuscular vitamin B12 with doses up to 1000micrograms 2-7 times per week. Treatment effect should be monitored frequently and the dose and frequency adjusted according to improvement in urinary MMA, plasma homocysteine and red cell indices. Hydroxocobalamin is effective and preferred over Cyanocobalamin for correcting defects in cobalamin metabolism such as CblC [42]. Mild dietary deficiency may be treated with oral vitamin B12; introduction of animal foods alone may not be inadequate. A longitudinal children fed a macrobiotic diet up to an average age of 6 years, before adopting a lacto-vegetarian or omnivorous diet showed at adolescence 21% still had elevated MMA concentrations, 15% had macrocytosis and 37% had low cobalamin concentrations (<218 pmol/L) [43]. Assessment of the cognitive performance of the same group of children in adolescence showed a significant association between cobalamin status (cobalamin<229 pmol/l or elevated urinary MMA>0.29 micromol/L) and deficits in cognitive function [44]. Due to the risk of adverse Neurodevelopmental outcomes, treatment with supplementation should be started as early as possible, there is evidence that long term outcome is related to duration and severity of deficiency.

Conclusion

The clinical features associated with vitamin B12 deficiency are non-specific, and hence can lead to a delay in diagnosis. Most clinical information on infantile B12 deficiency has been derived from clinical case reports with an average delay to diagnosis being 4 months [23].

The infant's brain is highly susceptible to permanent injury associated with vitamin B12 deficiency. The prevention of neurological damage is paramount on the initial clinical suspicion and timely treatment of B12 deficient states. The early clinical signs of infantile B12 deficiency are non-specific, and the haematological parameters traditional associated with B12 deficiency are a late phenomenon in infants. We propose that B12 deficiency should be considered in all infants with developmental delay and Hypotonia for whom an alternate diagnosis is not identified. Early identification and treatment can prevent irreversible brain injury and its profound associated impacts on the health of the child, their family, and their local health system.

References

1. Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Vitamins. December 2013.

2. Wright JD, Bialostosky K, Gunter EW, Carroll MD, Najjar MF, et al. (1998) Blood folate and vitamin B12: United States, 1988-94. Vital Health Stat 11: 1-78.

3. Allen LH, Rosado JL, Casterline JE, Martinez H, Lopez P, et al. (1995) Vitamin B-12 deficiency and malabsorption are highly prevalent in rural Mexican communities. Am J Clin Nutr 62: 1013-1019.

4. Kühne T, Bubl R, Baumgartner R (1991) Maternal vegan diet causing a serious infantile neurological disorder due to vitamin B12 deficiency. Eur J Pediatr 150: 205-208.

5. Rosenblatt DS, Whitehead VM (1999) Cobalamin and folate deficiency: acquired and hereditary disorders in children. Semin Hematol 36: 19-34.

6. Casterline JE, Allen LH, Ruel MT (1997) Vitamin B-12 deficiency is very prevalent in lactating Guatemalan women and their infants at three months postpartum. J Nutr 127: 1966-1972.

7. García-Casal MN, Osorio C, Landaeta M, Leets I, Matus P, et al. (2005) High prevalence of folic acid and vitamin B12 deficiencies in infants, children, adolescents and pregnant women in Venezuela. European Journal of Clinical Nutrition 59: 1064-1070.

8. Korenke GC, Hunneman DH, Eber S, Hanefeld F (2004) Severe encephalopathy with epilepsy in an infant caused by subclinical maternal pernicious anaemia: case report and review of the literature. Eur J Pediatr 163: 196-201.

9. Grange DK, Finlay JL (1994) Nutritional vitamin B12 deficiency in a breastfed infant following maternal gastric bypass. Pediatr Hematol Oncol 11: 311-318.

10. Monagle PT, Tauro GP (1997) Infantile megaloblastosis secondary to maternal vitamin B12 deficiency. Clin Lab Haematol 19: 23-25.

11. Graham SM, Arvela OM, Wise GA (1992) Long-term neurologic consequences in a breast-fed infant of a mother with pernicious anaemia. J Roy Soc Med 75: 656-658.

12. Fréry N, Huel G, Leroy M, Moreau T, Savard R, et al. (1992) Vitamin B12 among parturients and their newborns and its relationship with birthweight. Eur J Obstet Gynecol Reprod Biol 45: 155-163.

13. McPhee AJ, Davidson GP, Leahy M, Beare T (1988) Vitamin B12 deficiency in a breast fed infant. Arch Dis Child 63: 921-923.

14. Coman D, Bhattacharya K (2012) Extended newborn screening: an update for the general paediatrician. J Paediatr Child Health 48: E68-72.

15. Monsen AL, Refsum H, Markestad T, Ueland PM (2003) Cobalamin status and its biochemical markers methylmalonic acid and homocysteine in different age groups from 4 days to 19 years. Clin Chem 49: 2067-2075.

16. Campbell CD, Ganesh J, Ficicioglu C (2005) Two newborns with nutritional vitamin B12 deficiency: challenges in newborn screening for vitamin B12 deficiency. Haematologica 90: ECR45.

17. Marble M, Copeland S, Khanfar N, Rosenblatt DS (2008) Neonatal vitamin B12 deficiency secondary to maternal subclinical pernicious anemia: identification by expanded newborn screening. J Pediatr 152: 731-733.

18. Wiley V, Carpenter K, Wilcken B (1999) Newborn screening with tandem mass

spectrometry: 12 months' experience in NSW Australia. Acta Paediatr Suppl 88: 48-51.

19. Bjørke Monsen AL, Ueland PM, Vollset SE, Guttormsen AB, Markestad T, et al. (2001) Determinants of cobalamin status in newborns. Pediatrics 108: 624-630.

20. Allen LH (2002) Impact of vitamin B-12 deficiency during lactation on maternal and infant health. Adv Exp Med Biol 503: 57-67.

21. Specker BL, Black A, Allen L, Morrow F (1990) Vitamin B-12: low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic aciduria in their infants. Am J Clin Nutr 52: 1073-1076.

22. Zengin E, Sarper N, Caki Kiliç S (2009) Clinical manifestations of infants with nutritional vitamin B deficiency due to maternal dietary deficiency. Acta Paediatr 98: 98-102.

23. Dror DK, Allen LH (2008) Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutr Rev 66: 250-255.

24. Emery ES, Homans AC, Colletti RB (1997) Vitamin B12 deficiency: a cause of abnormal movements in infants. Pediatrics 99: 255-256.

25. Grattan-Smith PJ, Wilcken B, Procopis PG, Wise GA (1997) The neurological syndrome of infantile cobalamin deficiency: developmental regression and involuntary movements. Mov Disord 12: 39-46.

26. Hartmann H, Das AM, Lücke T (2011) Clinical presentation and metabolic consequences in 40 breastfed infants with nutritional vitamin B(12) deficiency-What have we learned? Eur J Paediatr Neurol 15: 377.

27. Baker SJ, Ignatius M, Johnson S, Vaish SK (1963) Hyperpigmentation of skin. A sign of vitamin-B12 deficiency. Br Med J 1: 1713-1715.

28. Gilliam JN, Cox AJ (1973) Epidermal changes in vitamin B 12 deficiency. Arch Dermatol 107: 231-236.

29. Duchen L, Jacobs J (1992). Nutritional deficiencies and metabolic disorders. In: Adams J, Duchen LW, eds. Greenfield's Neuropathology. New York, NY: Oxford University Press: 826-834.

30. Healton EB, Savage DG, Brust JC, Garrett TJ, Lindenbaum J (1991) Neurologic aspects of cobalamin deficiency. Medicine (Baltimore) 70: 229-245.

31. Lövblad K, Ramelli G, Remonda L, Nirkko AC, Ozdoba C, et al. (1997) Retardation of myelination due to dietary vitamin B12 deficiency: cranial MRI findings. Pediatr Radiol 27: 155-158.

32. Kunze K, Leitenmaier K (1976). Vitamin B12 deficiency and subacute combined degeneration of the spinal cord (funicular spinal disease). In: Vinken P, Bryun GW, eds. Handbook of Neurology. Vol 28. Amsterdam: Elsevier: 141-198.

33. Stollhoff K, Schulte FJ (1987) Vitamin B12 and brain development. Eur J Pediatr 146: 201-205.

34. Hashim G, Moscarello M (1983) Myelin structure, chemistry and immunology. In: Rosenberg R, ed. The Clinical Neurosciences. Vol 5. New York, NY: Churchill Livingstone 19-35.

35. Briddon A (2003) Homocysteine in the context of cobalamin metabolism and deficiency states. Amino Acids 24: 1-12.

36. Molloy A, Weir D (2001) Homocysteine and the nervous system. In: Jacobsen D, ed. Homocysteine in Health and Disease. Cambridge: Cambridge University Press 183-197.

37. Lipton SA, Kim WK, Choi YB, Kumar S, D'Emilia DM, et al. (1997) Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A 94: 5923-5928.

38. Guerra-Shinohara EM, Morita OE, Peres S, Pagliusi RA, Sampaio Neto LF, et al. (2004) Low ratio of S-adenosylmethionine to S-adenosylhomocysteine is associated with vitamin deficiency in Brazilian pregnant women and newborns. Am J Clin Nutr 80: 1312-1321.

39. Scalabrino G, Carpo M, Bamonti F, Pizzinelli S, D'Avino C, et al. (2004) High tumor necrosis factor-alpha [corrected] levels in cerebrospinal fluid of cobalamin-deficient patients. Ann Neurol 56: 886-890.

40. Scalabrino G, Peracchi M (2006) New insights into the pathophysiology of cobalamin deficiency. Trends Mol Med 12: 247-254.

41. Andersson HC, Shapira E (1998) Biochemical and clinical response to hydroxocobalamin versus cyanocobalamin treatment in patients with methylmalonic acidemia and homocystinuria (cblC). J Pediatr 132: 121-124.

42. van Dusseldorp M, Schneede J, Refsum H, Ueland PM, Thomas CM, et al. (1999) Risk of persistent cobalamin deficiency in adolescents fed a macrobiotic diet in early life. Am J Clin Nutr 69: 664-671.

43. Louwman MW, van Dusseldorp M, van de Vijver FJ, Thomas CM, Schneede J, et al. (2000) Signs of impaired cognitive function in adolescents with marginal cobalamin status. Am J Clin Nutr 72: 762-769.

44. von Schenck U, Bender-Götze C, Koletzko B (1997) Persistence of neurological damage induced by dietary vitamin B-12 deficiency in infancy. Arch Dis Child 77: 137-139.